

1	15	16	30	31	45	46	60	61	75	76	90
1 B.halodurans	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
2 C.acetobutylicum	-MNSKEMQKLQTS	YKEGWSCEIRVELQN	STRAHSISTAFDRK	DOGLKYETNLLERIL	SRENLIQALERVEKN	KSGSHGVDEMVKSLR	36				
3 Bst803-2065	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
4 PaicaligenesMatur	MPPVGAVSLVTVMQ	KEPTAETVIPNCPQK	PRVMPDSAKVPAASA	TWTNAEPDTLMERVL	APANLRRAYQVRVSN	KGAPGADGMTVADLA	90				
1 B.halodurans	105	106	120	121	135	136	150	151	165	166	180
2 C.acetobutylicum	LHLHENWTSIRNEII	EGSYFPPKPVRRVEIP	KPNGGVKRLGIPTVM	DRFLQQAIAQILTLQ	YDPTFSESFGRPH	RRGHNAVROAKQMMK	126				
3 Bst803-2065	QYLKQNGKTLIASIF	NGKYCPKAVRRVEIP	KPDGGIRLLGIPTVV	DRTIQQAISQVLTPI	FEKTFSENSYGFRRPK	RSAKQAIIKAKEYME	179				
4 PaicaligenesMatur	DYIRAHWSTIRAQLL	AGTYRPAVPVRRVGIP	KPGGGTROLGIPTVV	DRLIQQAIIQELTPI	FDPDFSPSFGFRPG	RNAHDAVROAQGYIQ	128				
	GYVKQYWPPTLKARLL	AGEYHPQAVRAVEIP	KPQGGTROLGIPSVV	DRLIQQAIIQQLTPI	FDPLFSDISYGFRRPG	RSTHQAIEMARAHVT	180				
1 B.halodurans	195	196	210	211	225	226	240	241	255	256	270
2 C.acetobutylicum	EGYRWVVVDIDLEKEF	DKVNHDRMLMRKLSR	IQDPRVLQILIRRYLQ	TGVMERGLVSPNTEG	TPQGGPLSPILLSNIV	LDELNDLEKRGKLF	216				
3 Bst803-2065	EGYKVVVDIDIDAKYF	DTVNHDKLMALVARK	IKDKRVLKILIRLYLQ	SGVMINGVVSETERG	CPQGGPLSPILLSNIM	LTELDRELEKRGHKF	269				
4 PaicaligenesMatur	EGYRYVVVDMDELEKEF	DRVNHDIILMSRVARK	VKDKRVLKILIRAYLQ	AGVMIEGVKVOTEEG	TPQGGPLSPILLANIL	LDDLDRELEKRGKLF	218				
	AGHRMCVDELDLEKEF	DRVNHDIILMACIERR	IKDKCVLRLIRRYLE	AGIMSGGVVSPROEG	TPQGGPLSPILLSNII	LDELRELERRGRHF	270				
1 B.halodurans	285	286	300	301	315	316	330	331	345	346	360
2 C.acetobutylicum	VRVADDNCNIYVRSKR	AGLRIMESVTSFTEN	RLKLKVNREKSADVDR	PWNRKFLGFSFTRGK	-DPKMRVSKESVKRL	KQRIRELTSSRRHSMK	305				
3 Bst803-2065	CRYADDNNVYVRSKK	AGDRVMRSITRFIEN	KLKLKVNREKSADVDR	PWNRKFLGFTFYQWY	GKIGIRVHEKSUVKF	KAKIKAITARNALN	359				
4 PaicaligenesMatur	CRYADDNCNIYVKSRL	AGQVVKOSIQRFLEK	TILKLKVNREKSADVDR	PWKRAFLGFSFTPER	-KARIRLAPRSIQRL	KQRIROLTNPNWSIS	307				
	VRVADDNCNIYVRSRPR	AGERVLVSVERFLRE	RLKLTVNRRKKSQVAVR	AWKCDYLGYGHSWHQ	-QPRLRVARMSLDRL	RDLRLMLRSVRARK	359				
1 B.halodurans	375	376	390	391	405	406	420	421	435	436	450
2 C.acetobutylicum	MSDRRLRLNRYLTGW	LGYQVVDTPSILAQ	IDAWTIRRLRMIRWK	EWKTTTSARQKNLVRL	GIKKAKAWQWANSRK	GYWRVAHSPIMDYAL	395				
3 Bst803-2065	IENRIIKLROCIIGW	LNIFYGIAETKLAKK	LDEWTIRRLRMCMYWK	QWKKKVTKYDNLKRF	GINNSKAWEFANTRK	SYWRANSFILSTTL	449				
4 PaicaligenesMatur	MPERIHRVNQYVMGW	IGYFRLVETPSVLQT	IEGWIRRLRLCQWL	QWKRVTRIRELRAL	GLKETAVMEIANTRK	GAWRITKTPQLHQAL	397				
	MATVIERINPVLRGW	ASYFKLSQSKRPLEE	LDGWVRHKLRCVIVR	QWKQPPTRLRLNMLRL	GLSEERANKSAFNGR	GPWNNSGAQHMYAL	449				

Fig. 1



B.

661 GATGTTGCGTGTGCAAGCAGAATTCTTTCCGAACTCATCTGAGGAAGCAAGGTGAAGC
 721 CCAGAGGGCCTCAGATCGAGGGCTGAGCGCAACCCGGCAAGACCTGAATCTCTCCCGC
 781 GAAGGAGAAGGAGAAGATCACG

803 atggcttctgttggaaacgcacatcttagcgagagacaacctcatcacg
 M A L L E R I L A R D N L I T
 848 gcgctcaaacgggtcgaagccaaccaaggagcaccgggaatcgac
 A L K R V E A N Q G A P G I D
 893 ggagatcaaccgatcaactccgtgattacatccgcgctcactgg
 G V S T D Q L R D Y I R A H W
 938 agcacgatccgcgcccaactcttggcgggaacctaccggccggcg
 S T I R A Q L L A G T Y R P A
 983 cctgtccgcagggtcggaatccgaaacgggcggcgccacacgg
 P . V . R . . R . . V . . G . . I . . F . . K . . P . . G . . G . . T . . R .
 1028 cagctaggcatccccaccgtggtgacgggtgatccaacaagcc
 Q L G I P T V V D R L I Q Q A
 1073 attcttcaagaactcacacccatttctgatccagacttctccct
 I L Q E L T P I F D P D F S P
 1118 tccagcttcggattccgtccggccgtaacgccacgatgccgtg
 S S F G F R P G R N A H D A V
 1163 cggcaagcgcaaggctacatccaggaagggtacggtacgtggtc
 R Q A Q G Y I Q E G Y R Y V V
 1208 gacatggacctggaagaagtcttctgatcgggtcaaccatgacatc
 D M D L E K F F D R V N H D I
 1253 ttgatgagtcgggtggccgaaagtcaaggataaacggtgctg
 L M S R V A R K V K D K R V L
 1298 aaactgatccgtgcctacctgcaagccggcgttatgatcgaagg
 K L I R A Y L Q A G V M I E G
 1343 gtgaagggtgcagacggaggaaggacgcgcgaaggcggcccttc
 V K V Q T E E G T P Q G G P L
 1388 agcccccctgctggcgaacatccttctgcacgatttagacaaggaa
 S P L L A N I L D D L D K E
 1433 ttggagaagcgaggattgaaattctgcccgttacgcagatgactgc
 L E K R G L K F C R Y A D D C
 1478 aacatctatgtgaaaagtctgcccgaagacaacgggtgaaacaa
 N I Y V K S L R A G Q R V K Q
 1523 agcatccaacgggtcttggagaaaacgctcaaaactcaaaagtaaac
 S I Q R F L E K T L K L K V N
 1568 gaggagaaaagtgcgggtggaccgccgtggaaacgggcctttctg
 E E K S A V D R P W K R A F L
 1613 gggtttagcttcacaccggaacgaaaagcgcgaatccggctcgcc
 G F S F T P E R K A R I R L A
 1658 ccaaggtcgattcaacgtctgaaacagcggattcgacagctgacc
 P R S I Q R L K Q R I R Q L T
 1703 aacccaaactggagcatatcgatgccagaacgaattcatcgctc
 N P N W S I S M P E R I H R V
 1748 aatcaatacgtcatgggatggatcggtattttcggtcgtcgaa
 N Q Y V M G W I G Y F R L V E
 1793 accccgtctgtccttcagaccatcgaaggatggattcggaggagg
 T P S V L Q T I E G W I R R R
 1838 cttcgactctgtaaatggcttcaatggaaacgggtcagaaccaga
 L R L C Q W L Q W K R V R T R
 1883 atccgtgagttaaagagcgtgggctgaaagagacagcgggtgatg
 I R E L R A L G L K E T A V M
 1928 gagatcgccaatacccgaaggagcttggcgaaacagaaacg
 E I A N T R K G A W R T T K T
 1973 ccgcaactccaccagccctggcgaaacactactggaccgctcaa
 P Q L H Q A L G T Y W T A Q
 2018 gggctcaagagtttgacgcaacgatatttcgaactcgtcaaggt
 G L K S L T Q R Y F E L R Q G
 2063 tga

Bst755

Bst1396

Bst2015

Bst2198

CGAACCCGCTAGTCGGACCCGATGCTAGGTGGT
 2102 GTGAGGGGACGGGGTTAGCCGCCCCCTCCTACTCGATTGCTATTGCTATTCGGCGCTAT
 2162 GCCACGCGAAACGGCCATGAACGTCAAGCCCTTCTCCTTGTAGATCGTCTCCTTCCCGC
 2222 GCACGCCGTTGATCGAATAGCTCGCTGTAATGGCGCATTAAACGAATGGGAAACGGAAC

Fig. 2

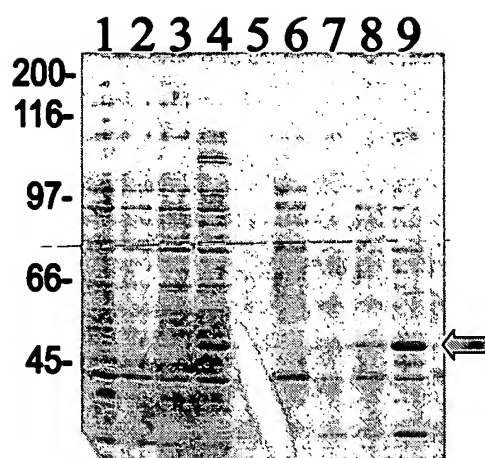


Fig. 3

BEST AVAILABLE COPY

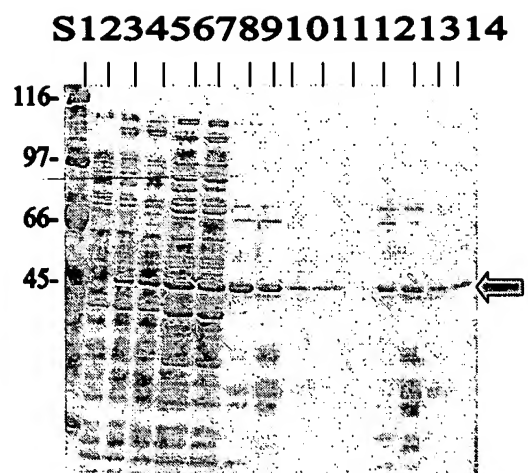


Fig. 4

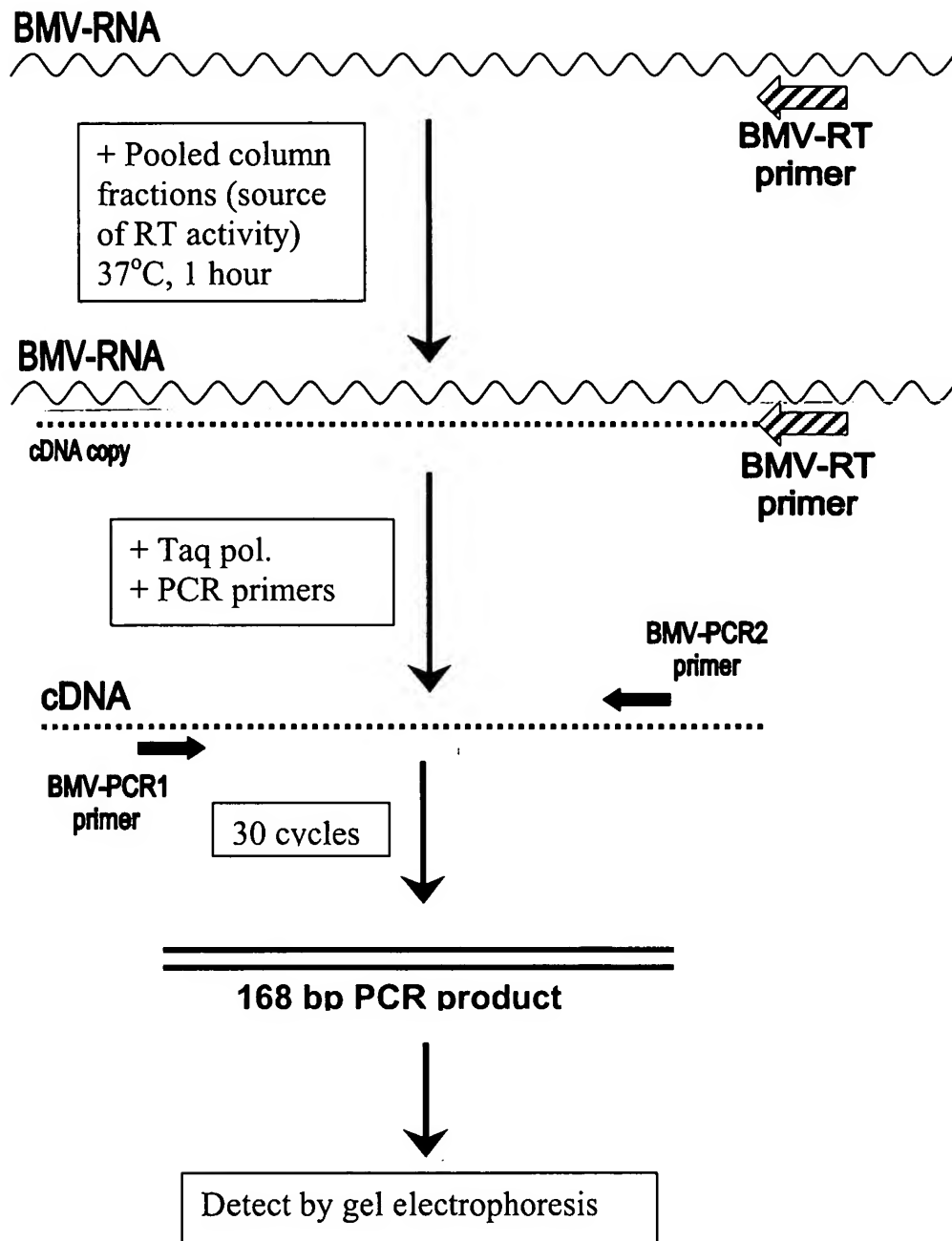


Fig. 5

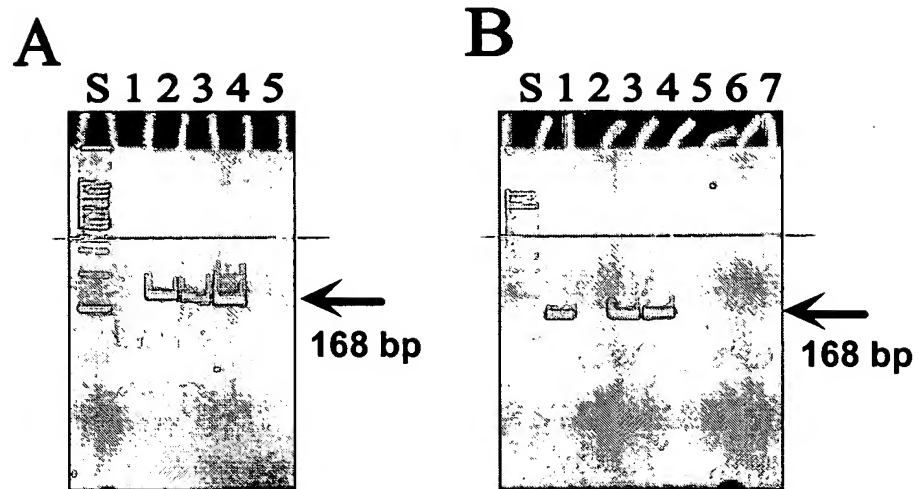


Fig. 6

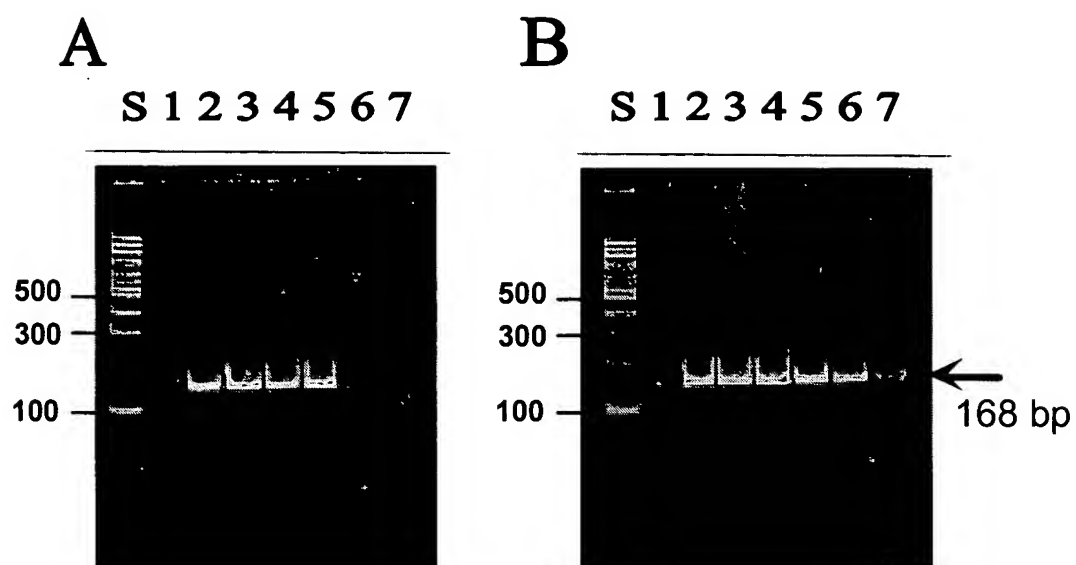
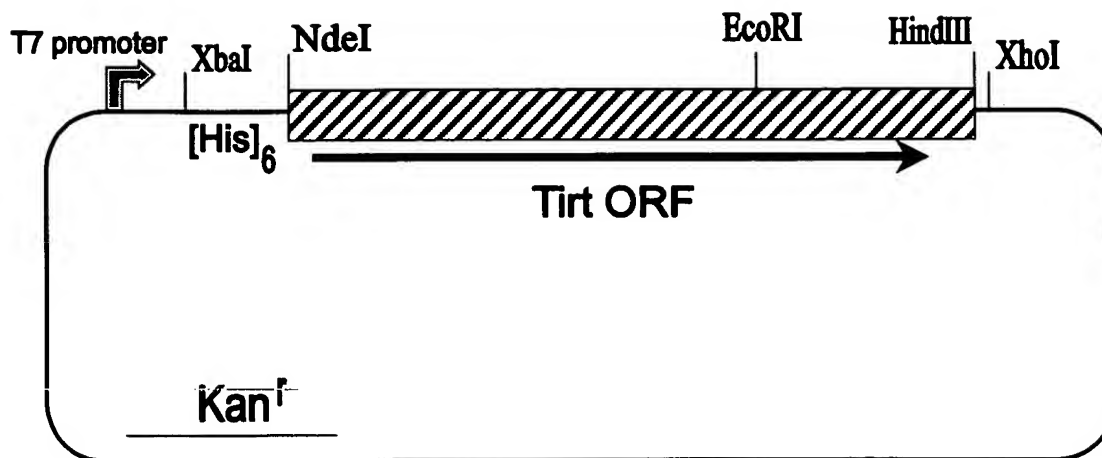


Fig. 7

A.



Plasmid pTirt#16

B.

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                                T7 promoter
1  TGGNNNNAGT NTTTAAACCT TTNACCGCC NTAATACNAC TCACTATAGG
   lac operator
51  GGAATTGTGA GCGGATAACA ATTCCCTCT AGAAATAATT TTNTTAACT
   rbs                                His-tag
101 TTAAGAAGGA GATATACCat gggcagcagc catcatcatc atcatcacag
      M   G S S   H H H H   H H S
      > start of fusion NdeI
151 cagcggcctg gtgccgcgcg gcagccatat gcggcaagac ctgaatctca
      S G L   V P R G   S H M   R Q D   L N L I
201 tcccgcggaa ggagaagatc acgatggctt tggtggaacg catcttagcg
      P R K   E K I   T M   A L   L E R   I L A
      > start of Tirt ORF
251 agagacaacc tcatcacggc gctcaaacgg gtcgaagcca accaaggagc
      R D N L   I T A   L K R   V E A N   Q G A
  
```

Fig. 8